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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : G01N 33/569, 33/68

A1

(11) International Publication Number: **WO 98/08096**

(43) International Publication Date: 26 February 1998 (26.02.98)

(21) International Application Number: PCT/NL97/00473

(22) International Filing Date: 20 August 1997 (20.08.97)

(30) Priority Data:
1003839 20 August 1996 (20.08.96) NL

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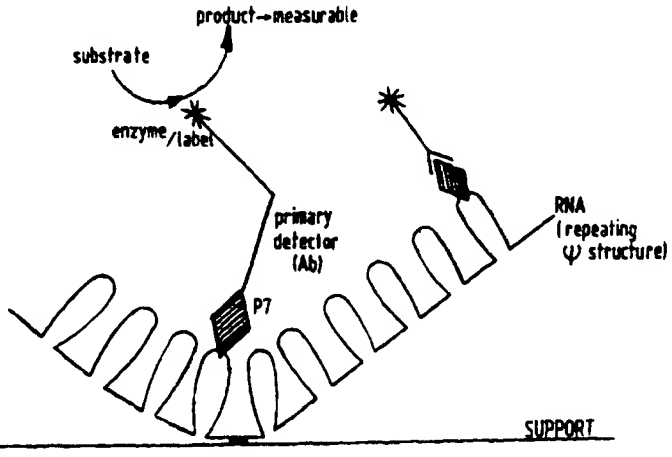
(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(54) Title: DIAGNOSTIC TEST



(57) Abstract

The invention relates to a method and kit for detecting nucleic acid-binding molecules, in particular proteins, in a sample of for instance a body fluid. The method comprises causing the molecule for detecting to bind to a first ligand and detecting the binding between the molecule and the first ligand by binding a second ligand to the molecule, wherein at least one of the two ligands is a nucleic acid. According to the invention there are three general embodiments, i.e. that wherein the first ligand is a nucleic acid, that wherein the second ligand is a nucleic acid and that wherein both ligands are nucleic acids.

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DIAGNOSTIC TEST

The present invention relates to diagnostic methods particularly intended for detecting nucleic acid-binding molecules, such as proteins. The invention further relates to kits for performing the method.

Diagnostic tests usually make use of the specific binding capacity of antibodies to detect the presence of a particular molecule in a sample. The best known diagnostic tests are the ELISA and the EIA or RIA. To detect the presence of antibodies to a known antigen, the antigen is coupled in an ELISA to a solid carrier such as a microtitre plate. By bringing the sample into contact with the carrier, antibodies which are specific to the antigen will bind thereto. After removing non-bound sample material and labelling the bound antibody the binding can be visualized and optionally quantified.

When the presence of an antigen in the sample must be detected use is often made of a so-called sandwich ELISA. Herein an antibody which is specific to the antigen for detecting is bound to a solid carrier. After contact with the sample and removal of non-bound sample material the antigen bound to the primary antibody is detected with a secondary antibody directed against another epitope on the molecule. The utility of this method does however require the presence of at least two different, sufficiently immunogenic epitopes. Some molecules for detecting are however too small or insufficiently immunogenic to enable detection in this manner.

Such a molecule is for instance the p7-protein of human immunodeficiency virus 1 (HIV-1). P7, also called NCP7 or nucleocapsid, is one of the four proteins which together form the nucleocapsid of the virus. They originate from one 55 kDa protein (Pr55) which is coded by the gag gene of HIV. P7 consists of 55 or 72 amino acids and the mass is about 7 kDa. It binds, in principle at random, to a piece of nucleic acid of a minimum of 6 bases long in any sequence, thus for instance pieces of nucleic

acid of a minimum of 6 bases long distributed over the entire HIV RNA, but with a higher specificity to the so-called ψ-structure. This is one of the hairpin structures at the beginning of the HIV-1 RNA which are probably involved in the coupling between the two RNA molecules of a virus particle.

Because p7 is relatively small it is also not very immunogenic. This makes detection of the protein by means of antibodies more difficult. The normal diagnostic tests are therefore not suitable for detecting the presence of p7 in a sample.

It is an object of the present invention to provide a diagnostic test for detecting (small or not very immunogenic) nucleic acid-binding molecules in general and p7 in particular in samples, particularly of bodily fluids.

This is achieved by the invention with a method comprising of causing the molecule for detecting to bind to a first ligand and detecting the binding between the molecule and the first ligand by binding a second ligand to the molecule, wherein at least one of the two ligands is a nucleic acid formed by a minimum of 3 bases. In this text "nucleic acid" is understood to mean in each case a piece of nucleic acid having a length of a minimum of 3 bases in random sequence.

The basic method according to the invention has three general embodiments, each in turn comprising a number of specific embodiments. It is thus possible to use a nucleic acid for binding and other means, in particular an antibody, for detection, but it is also possible to use an antibody for binding and a nucleic acid for detection. Finally, both binding and detection can be realized by means of nucleic acids.

The first embodiment comprises of:

a) bringing the bodily fluid into contact with a carrier having as first ligand at least one nucleic acid bound thereto in order to enable binding between the molecule for detecting and the nucleic acid,

b) making visible the binding between the nucleic acid and the nucleic acid-binding molecule by means of at

least one antibody directed against the molecule as second ligand, and

c) visualizing and optionally quantifying the binding.

This method is illustrated in Fig. 1 with p7 as protein for detecting.

In the second embodiment the functions of the nucleic acid and the antibody are exchanged. This method comprises of:

a) bringing the bodily fluid into contact with a carrier having as first ligand at least one antibody bound thereto directed against the molecule for detecting in order to enable binding between the molecules and the antibody,

b) making visible the binding between the antibody and the nucleic acid-binding molecule by means of at least one nucleic acid binding the molecule as second ligand, and

c) visualizing and optionally quantifying the binding.

Fig. 2 gives a schematic view of this method.

The third embodiment (shown in Fig. 3) relates to a method, wherein nucleic acids are used for both functions (binding and detection), comprising of:

a) bringing the bodily fluid into contact with a carrier having as first ligand at least one nucleic acid in order to enable binding between the molecule and the nucleic acid,

b) making visible the binding between the nucleic acid and the nucleic acid-binding molecule by means of at least one nucleic acid binding the molecule as second ligand, and

c) visualizing and optionally quantifying the binding.

Other than in the sandwich ELISA, the primary binding of the molecule for detecting to the carrier is effected according to the first embodiment of the invention by means of a nucleic acid. The secondary binding (detection) does still occur in this first embodiment via an antibody. The advantage is however that the molecule

for detecting only has to have one epitope to enable detection. The method according to the second embodiment also has the above stated advantage of only one epitope being required. It is even the case in the third embodiment that epitopes are no longer required at all. The only condition is that the molecule for detecting binds to the nucleic acids used, but this condition does of course apply to all embodiments.

The nucleic acid can be RNA, single-stranded DNA or double-stranded DNA. The sequence of the nucleic acid preferably corresponds at least partly with that of naturally occurring nucleic acid, such as HIV-1 RNA, wherein modifications which do not have a negative effect on the binding capacity of the nucleic acid to the molecule for detecting are permitted.

Modifications can be sequence modifications or chemical modifications. Thus, in addition to naturally occurring nucleic acids or synthetic homologies thereof, sequence-modified versions can also be used. This is understood to mean in this case any molecule which does not occur in nature but which has advantageous properties for the test. As an example can be mentioned a repeating sequence of the components relevant for binding from a naturally occurring nucleic acid. For p7 this could for instance be a sequence of repeating ψ-structures.

Chemical modification may be necessary to protect the nucleic acid against degradation by nucleic acid-degrading enzymes from the sample, such as RNase or DNase. Presence of these enzymes in the sample could render the test unusable. Examples of such modifications protecting against degradation are the complexing of the RNA with vanadium.

Protection against nucleic acid-degrading enzymes can also be effected by adding one or more RNase- or DNase-inhibiting means to the sample or to the carrier before placing the sample into contact with the carrier with the nucleic acid.

The nucleic acid for detection according to the third embodiment can bind specifically to the molecule

for detecting, but can also have very general (protein) binding properties.

The carrier can take different forms, such as a microtitre plate, a column, a membrane or beads. These latter may for instance be magnetic beads such as Dynabeads™.

In the first embodiment of the invention the binding of the antibody to the molecule for detecting can be detected in different ways. The method which is particularly recommended is labelling the antibody with a radioactive or fluorescent label or with an enzyme, which can result in a staining reaction, such as horseradish peroxidase. A method which works via an electrochemiluminescent label is possible if the antibodies or the nucleic acids necessary for detection are labelled with an electrochemiluminescent label, wherein the systematics of detection are the same as that of the NASBA RNA (Organon Technika, Oss). This substance can be energetically excited by an intermediary substance after electron transfer of both the electrochemiluminescent label and the intermediary substances has taken place via an electrode in the analyzer. When the label thereafter falls back again to a normal energy level, a determined quantity of energy is released, in the form of a photon. These photons can be detected and measured in a so-called photon multiplier tube and can be quantified.

In the case of the second and the third embodiment of the invention the nucleic acid for detection is labelled. The same labels can herein be used as for labelling an antibody for detection.

In addition to a complete antibody, antibody fragments such as Fv, Fab, $_{F}$(ab)2, chimeric or bispecific antibodies can also be used.

To the skilled person in this field it is quite simple to isolate or synthesize a suitable nucleic acid on the basis of his knowledge of the molecule for detecting. Production of the antibody for primary binding or detection is also a routine matter for a skilled person and is for instance described in: "Antibodies, a laboratory manual" (Harlow and Lane, Cold Spring Harbor Labora-

tory (1988). Binding of nucleic acids to different carriers can be performed via a streptavidin-biotin binding. Carriers can be coated with streptavidin and nucleic acid can be labelled with biotin via the incorporation of a biotin-labelled nucleoside triphosphate during the synthesis of the nucleic acid. After the carrier has been coated with streptavidin, biotin-labelled nucleic acid is added and the binding takes place. Binding of the antibody for primary binding to the carrier is standard technique and is described for instance in Laboratory techniques in biochemistry and molecular biology. Practice and theory of enzyme immuno-assays., P. Tijssen, Elsevier Amsterdam 1987. Labelling of the antibody for detection is also a per se known technique (Tijssen, supra).

Labelling of nucleic acids for detection can take place as follows. The nucleoside triphosphate GTP lends itself to labelling with an enzyme or label which is coupled to an N-hydroxysuccinimide ester. The free amine group on this nucleoside is not used for hybridization of the nucleic acids to each other via hydrogen bridges and can thus be labelled. Another possibility is to label the nucleic acid at the 5'-terminal end with an amino linker, so that the activated enzyme or label can react and bind herewith.

In addition to the methods the invention further provides diagnostic kits for performing thereof. Very generally a basic kit comprises a carrier with a first ligand bound thereto for binding the molecule for detecting and a second ligand for binding the molecule to detect the binding between the molecule and the first ligand, wherein at least one of the two ligands is a nucleic acid.

In the first general embodiment the kit comprises a carrier having as first ligand at least one nucleic acid bound thereto and means for detecting binding of the nucleic acid with the nucleic acid-binding molecule for detecting. In a more specific embodiment the diagnostic kit comprises a microtitre plate as carrier for the nucleic acid and means for detecting binding of the

nucleic acid with the nucleic acid-binding molecule for detecting. Which means these may be has already been described above for the method. The means for detection are preferably (labelled) antibodies.

In another specific embodiment the diagnostic kit comprises magnetic beads as carrier for the nucleic acid and antibodies labelled for chemiluminescence as the means for detecting binding of the nucleic acid to the nucleic acid-binding molecule for detecting.

In a still more specific embodiment the diagnostic kit is intended to detect the presence of HIV-1 in a bodily fluid, and comprises Dynabeads™ with a nucleic acid bound thereto, consisting of a repeating sequence of the p7-binding psi-structure of HIV-1, and a (monoclonal) antibody labelled for chemiluminescence directed against p7 for the detection of p7 protein bound to the nucleic acid.

Kits according to second general embodiment of the invention comprise a carrier having as first ligand at least one antibody bound thereto directed against the molecule for detecting and as second ligand a nucleic acid binding the molecule for detecting in order to detect binding of the antibody with the nucleic acid-binding molecule for detecting.

Kits according to the third general embodiment comprise a carrier having as first ligand at least one first nucleic acid bound thereto which can bind to the molecule for detecting, and as second ligand a second nucleic acid binding the molecule for detecting and labelled for detection in order to detect binding of the first nucleic acid to the nucleic acid-binding molecule for detecting.

All kits can moreover contain means for inhibiting nucleic acid-degrading enzymes potentially present in the sample.

The methods and kits according to the invention are particularly suitable for testing different bodily fluids, such as blood, serum, plasma, sperm, urine, saliva, tear water, liquor or sweat.

The present invention can have a wide diversity of applications. In the examples however, only one specific embodiment for detecting p7 in serum will be discussed. It will be apparent to the skilled person that the invention is very general and does not lie in this specific embodiment. Using his average professional knowledge the skilled person in this field will be able to apply the principle of the invention, i.e. detecting a molecule by making use of the capacity of that molecule to bind to nucleic acid. This can take place by primary binding to a nucleic acid and detection by means of secondary binding to an antibody, by primary binding to an antibody and detection by secondary binding to a nucleic acid or by primary as well as secondary binding to a nucleic acid. The examples hereinbelow are therefore only given by way of illustration.

EXAMPLES

EXAMPLE 1

Diagnostic test on the basis of microtitre plate

A 96-well microtitre plate was coated with complete HIV-1 RNA by incubating virus-purified RNA in a concentration of 10^5 molecules in 100 μl demineralized and autoclaved water overnight at room temperature. The following day the wells were washed with an autoclaved washing buffer, consisting of 25 μg/ml yeast tRNA, 200 mM KCl and 40 mM $MgCl_2$ in dH_2O.

25 μl serum sample with 100 μl washing buffer and 8 serum samples with a known quantity of HIV-1 p7 was subsequently placed in the wells in triplicate. After 45 minutes incubation at room temperature the sample was removed and the wells were washed with washing buffer. Hereafter 100 μl anti-HIV-1 p7 antibody labelled with horseradish peroxidase (manufactured as described in "Antibodies, a laboratory manual", supra) was placed in the wells in a concentration of 2 μg/ml. After incubation for one hour at 37°C the antibody solution was removed and the wells were washed with washing buffer.

The protein for detecting was then quantified by placing 100 μl substrate solution (OPD) in the wells. The staining reaction which subsequently occurred was stopped

after 30 minutes by adding 50µl of a 0.5M sulphuric acid solution. The optical density of the content of the wells was subsequently measured at 450 nm in a spectrophotometer. The values of the 8 calibration samples were subsequently plotted against the known concentration of p7, whereby a calibration line resulted and the p7 concentration in the serum samples could be determined by reading the value on the calibration line.

EXAMPLE 2

Diagnostic test on the basis of Dynabeads™

12x75 mm tubes with a round bottom were filled with 25 µl biotin-labelled RNA in dilution buffer (25 µg/ml yeast tRNA, 200 mM KCl, 40 mM $MgCl_2$ and 5U/ml RNasin (Promega) in dH_2O), 25µl of a solution of an anti-HIV-1 p7 antibody (2 µg/ml) labelled with an electrochemiluminescent label, and 25 µl serum sample. A calibration line was made by replacing the serum samples to be tested by 25 µl serum samples with a known quantity of p7. After 30 minutes at room temperature with continuous shaking, 2.5 µg Dynabeads™ (M-280, coated with streptavidin) in 25 µl dilution buffer was added. After 15 minutes incubation at room temperature with continuous shaking, 200 µl assay buffer (IGEN Inc., Gaithersburg, MD, USA) was added thereto and the samples were analysed in the ORIGEN-analyzer (IGEN Inc., Gaithersburg, MD, USA).

EXAMPLE 3

Diagnostic test with sequence-modified nucleic acid

The test was performed in the same manner as in Example 2, but instead of complete RNA a synthetic RNA was used which consisted of 10 times in succession the sequence of HIV-1_MN nucleotides 300 up to and including 337, which sequence contains the ψ-hairpin.

EXAMPLE 4

Diagnostic test with chemically modified nucleic acid

In order to prevent degradation of the nucleic acid by RNase, the same RNA as in Example 3 was complexed with vanadyl sulphate in accordance with the protocol descri-

bed in Maniatis, Fritsc and Sambrook, Molecular Cloning, a laboratory manual, Cold Spring Harbor Laboratory, 1982.

EXAMPLE 5

Diagnostic test with nucleic acid as detection agent

Examples 1 to 4 related to diagnostic tests, wherein the molecule for detecting was primarily bound by a nucleic acid and secondarily detected by means of an antibody. In this example the reverse case is described, wherein primary binding takes place by means of an antibody and detection by means of a labelled nucleic acid.

A 96-well microtitre plate was coated with anti-HIV-1 p7 antibody (prepared as described in "Antibodies, a laboratory manual", supra) by incubating it with 100 µl of an antibody solution with a concentration of 2 µg/ml in demineralized and autoclaved water overnight at room temperature. The following day the wells were washed with an autoclaved washing buffer, consisting of PBS/0.5% Tween-20™.

25 µl serum sample with 100 µl washing buffer and 8 serum samples with a known quantity of HIV-1 p7 was subsequently placed in the wells in triplicate. After 60 minutes incubation at room temperature the sample was removed and the wells were washed with 25 µg/ml yeast tRNA, 200 mM KCl, 40 mM $MgCl_2$ and 5 U/ml RNasin (Promega) in dH_2O. Hereafter 100 µl of a solution of the nucleic acid labelled with horseradish peroxidase from Example 3 was placed in the wells in a concentration of 10^5 nucleic acid molecules per 100 µl. After 30 minutes incubation at room temperature the nucleic acid solution was removed and the wells were washed with 25 µg/ml yeast tRNA, 200 mM KCl, 40 mM $MgCl_2$ and 5 U/ml RNasin (Promega) in dH_2O.

The protein for detecting was subsequently quantified by placing 100 µl substrate solution (OPD) in the wells. The staining reaction which subsequently occurred was stopped after 30 minutes by adding 50 µl 0.5 M H_2SO_4. The optical density of the content of the wells was then measured at 450 nm in a spectrophotometer. The values of the 8 calibration samples were subsequently plotted against the known concentration of p7, whereby a calibra-

tion line resulted and the p7 concentration in the serum samples could be determined by reading the value on the calibration line.

EXAMPLE 6

Diagnostic kit with nucleic acids for both binding and detection

In a third alternative embodiment according to the invention both primary binding and secondary binding for detection are effected by means of nucleic acids. This example illustrates this embodiment.

A 96-well microtitre plate was coated with complete HIV-1 RNA by incubating virus-purified RNA in a concentration of 10^5 molecules in 100 μl demineralized and autoclaved water overnight at room temperature. The following day the wells were washed with a autoclaved washing buffer, consisting of 25 μg/ml yeast tRNA, 200 mM KCl and 40 mM $MgCl_2$ in dH_2O.

25 μl serum sample with 100 μl washing buffer and 8 serum samples with a known quantity of HIV-1 p7 was subsequently placed in the wells in triplicate. After 45 minutes incubation at room temperature the sample was removed and the wells were washed with washing buffer.

Hereafter 100 μl of a solution of the nucleic acid labelled with horseradish peroxidase from Example 3 was placed in the wells in a concentration of 10^5 molecules per 100 μl. After 45 minutes incubation at room temperature the nucleic acid solution was removed and the wells were washed with washing buffer as described above.

The protein for detecting was subsequently quantified by placing 100 μl substrate solution (OPD) in the wells. The staining reaction which then occurred was stopped after 30 minutes by adding 50 μl 0.5 M H_2SO_4. The optical density of the content of the wells was subsequently measured at 450 nm in a spectrophotometer. The values of the 8 calibration samples were subsequently plotted against the known concentration of p7, whereby a calibration line resulted and the p7 concentration in the serum samples could be determined by reading the value on the calibration line.

CLAIMS

1. Method for detecting nucleic acid-binding molecules, in particular proteins, in a sample of for instance a bodily fluid, comprising of causing the molecule for detecting to bind to a first ligand and detecting the binding between the molecule and the first ligand by binding a second ligand to the molecule, wherein at least one of the two ligands is a nucleic acid.

2. Method as claimed in claim 1, comprising of:

a) bringing the bodily fluid into contact with a carrier having as first ligand at least one nucleic acid bound thereto in order to enable binding between the molecule for detecting and the nucleic acid,

b) making visible the binding between the nucleic acid and the nucleic acid-binding molecule by means of at least one antibody directed against the molecule as second ligand, and

c) visualizing and optionally quantifying the binding.

3. Method as claimed in claim 1, comprising of:

a) bringing the bodily fluid into contact with a carrier having as first ligand at least one antibody bound thereto directed against the molecule for detecting in order to enable binding between the molecule and the antibody,

b) making visible the binding between the antibody and the nucleic acid-binding molecule by means of at least one nucleic acid binding the molecule as second ligand, and

c) visualizing and optionally quantifying the binding.

4. Method as claimed in claim 1, comprising of:

a) bringing the bodily fluid into contact with a carrier having as first ligand at least one nucleic acid in order to enable binding between the molecule and the nucleic acid,

b) making visible the binding between the nucleic acid and the nucleic acid-binding molecule by means of at least one nucleic acid binding the molecule as second ligand, and

c) visualizing and optionally quantifying the binding.

5. Method as claimed in any of the claims 1-4, **characterized in that** the nucleic acid is formed by RNA and/or single-stranded DNA and/or double-stranded DNA or modified versions thereof.

6. Method as claimed in any of the claims 1-5, **characterized in that** the sequence of the nucleic acid corresponds at least partly with that of naturally occurring HIV-1 RNA, wherein modifications which do not have a negative effect on the binding capacity of the nucleic acid to the molecule for detecting are permitted.

7. Method as claimed in claim 6, **characterized in that** the nucleic acid-binding molecule for detecting is HIV-1 p7 and the nucleic acid contains at least one HIV-1 p7 binding sequence.

8. Method as claimed in any of the claims 1-7, **characterized in that** the bodily fluid is blood, serum, plasma, sperm, urine, saliva, tear water, liquor or sweat.

9. Diagnostic kit for performing the method as claimed in any of the claims 1-8, comprising a carrier with a first ligand bound thereto for binding the molecule for detecting and a second ligand for binding the molecule in order to detect the binding between the molecule and the first ligand, wherein at least one of the two ligands is a nucleic acid.

10. Diagnostic kit as claimed in claim 9 for performing the method as claimed in any of the claims 1, 2 and 5-8, comprising a carrier having as first ligand at least one nucleic acid bound thereto and as second ligand means for detecting binding of the nucleic acid with the nucleic acid-binding molecule for detecting.

11. Diagnostic kit as claimed in claim 10, **characterized in that** the carrier for the nucleic acid is a microtitre plate and the means for detecting binding of

the nucleic acid with the nucleic acid-binding molecule for detecting are antibodies.

12. Diagnostic kit as claimed in claim 10, **characterized in that** the carrier for the nucleic acid is formed by magnetic beads and the means for detecting binding of the nucleic acid to the nucleic acid-binding molecule for detecting are antibodies labelled for chemiluminescence.

13. Diagnostic kit as claimed in claim 12 for detecting the presence of HIV-1 in a bodily fluid, comprising Dynabeads™ having as first ligand a nucleic acid bound thereto and consisting of a repeating sequence of the p7-binding psi-structure of HIV-1, and a monoclonal antibody labelled for chemiluminescence directed against p7 as second ligand in order to detect p7 protein bound to the nucleic acid.

14. Diagnostic kit as claimed in claim 9 for performing the method as claimed in any of the claims 1, 3 and 5-8, comprising a carrier having as first ligand at least one antibody bound thereto and directed against the molecule for detecting and as second ligand a nucleic acid binding the molecule for detecting and labelled for detection in order to detect binding of the antibody with the nucleic acid-binding molecule for detecting.

15. Diagnostic kit as claimed in claim 9 for performing the method as claimed in any of the claims 1 and 4-8, comprising a carrier having as first ligand at least one first nucleic acid bound thereto which can bind to the molecule for detecting, and as second ligand a second nucleic acid binding the molecule for detecting and labelled for detection in order to detect binding of the first nucleic acid to the nucleic acid-binding molecule for detecting.

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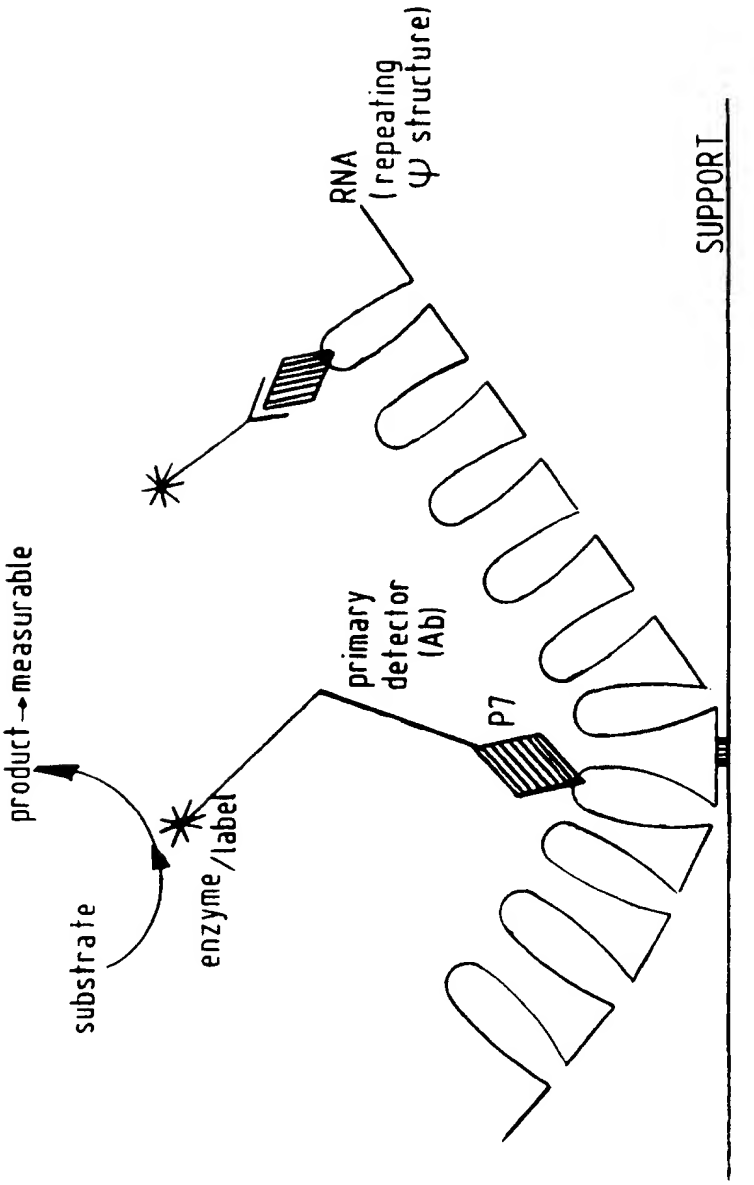
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(Ab)
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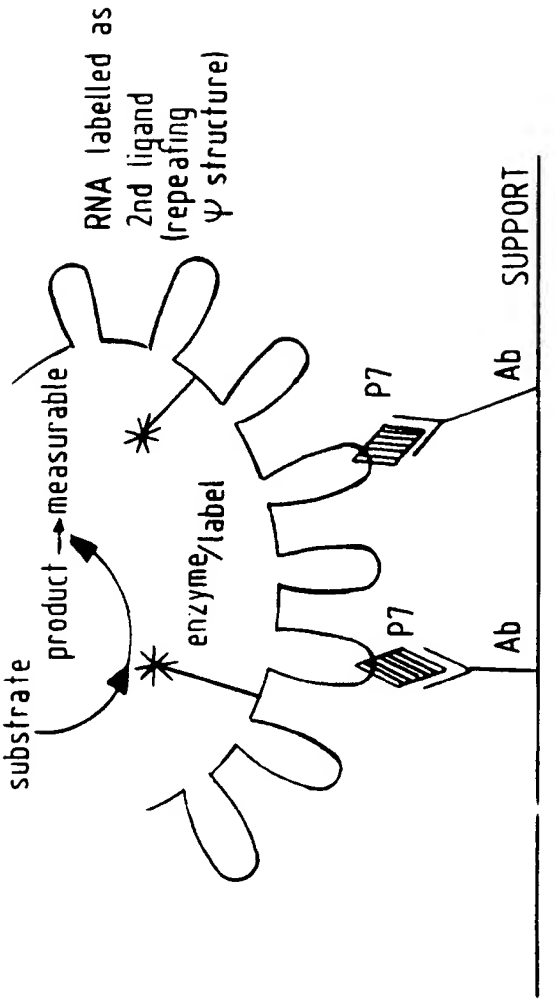
product→measurable
enzyme/label
substrate

FIG.1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P7
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Ab
SUPPORT

FIG.2

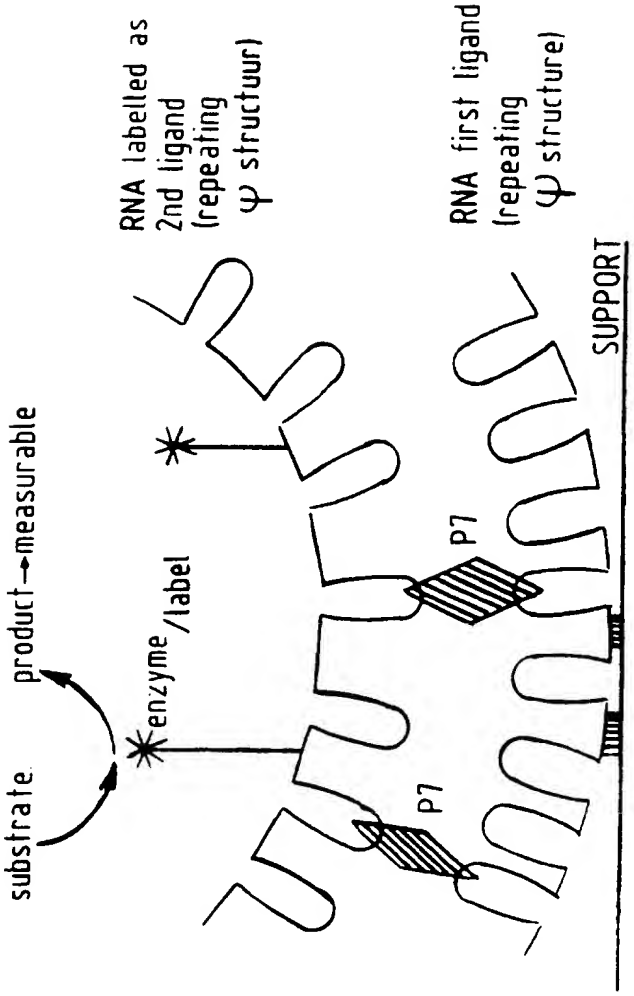
substrate
product→measurable
enzyme/label
RNA labelled as 2nd ligand (repeating ψ structuur)
RNA first ligand (repeating ψ structure)
P7
P7
SUPPORT

FIG.3

INTERNATIONAL SEARCH REPORT

Inte tional Application No
PCT/NL 97/00473

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 G01N33/569 G01N33/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	HIBMA, MERILYN H. ET AL: "A nonradioactive assay for the detection and quantitation of a DNA - binding protein" NUCLEIC ACIDS RES. (1994), 22(18), 3806-7 CODEN: NARHAD;ISSN: 0305-1048, 1994, XP000673300 see page 3806, left-hand column, last paragraph - page 3807, left-hand column, paragraph 1	1,2,4,5, 8-12,14, 15
Y	see the whole document	3,6,7,13
X	EP 0 620 439 A (BOEHRINGER MANNHEIM GMBH) 19 October 1994 see claims	1,2,4,5, 8-12,14, 15

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
7 October 1997	20 -10- 1997

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx 31 651 epo nl, Fax: (+31-70) 340-3016	Hoekstra, S

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INTERNATIONAL SEARCH REPORT

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	NOVAK, ULRIKE ET AL: "Identification of proteins in DNA-protein complexes after blotting of EMSA gels" BIOTECHNIQUES (1995), 19(1), 54-5 CODEN: BTNQDO;ISSN: 0736-6205, 1995, XP000673308 see the whole document ---	1
Y	US 5 462 852 A (ARTHUR, LARRY O. ET AL) 31 October 1995 see column 2, last paragraph - column 4, paragraph 4 -----	3,6,7,13

INTERNATIONAL SEARCH REPORT

Inte tional Application No
PC1/NL 97/00473

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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US 5462852 A	31-10-95	NONE	

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